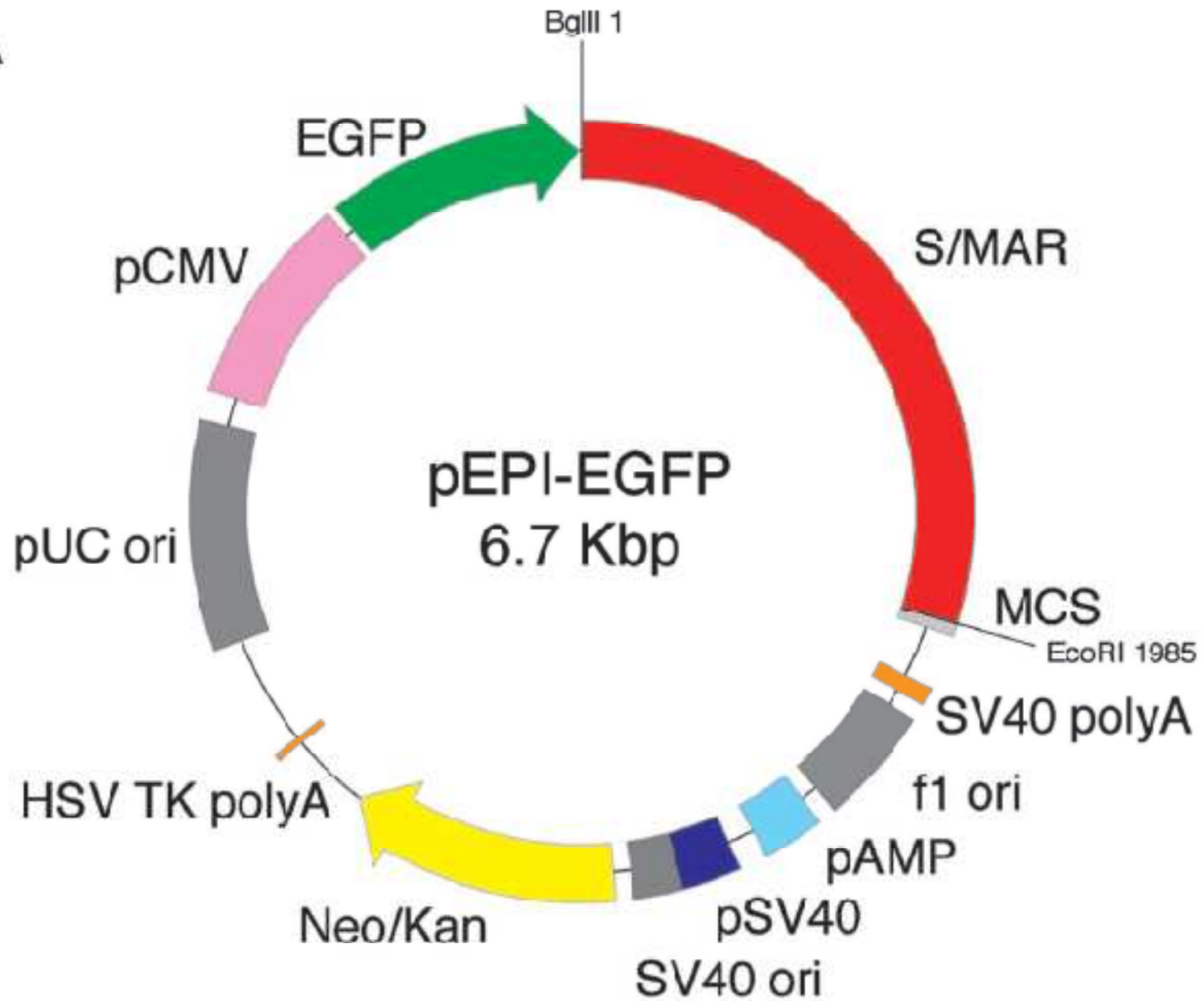


Figure 1
A scheme for *Tol2*-mediated BAC transgenesis. (a)

A



Research Reports

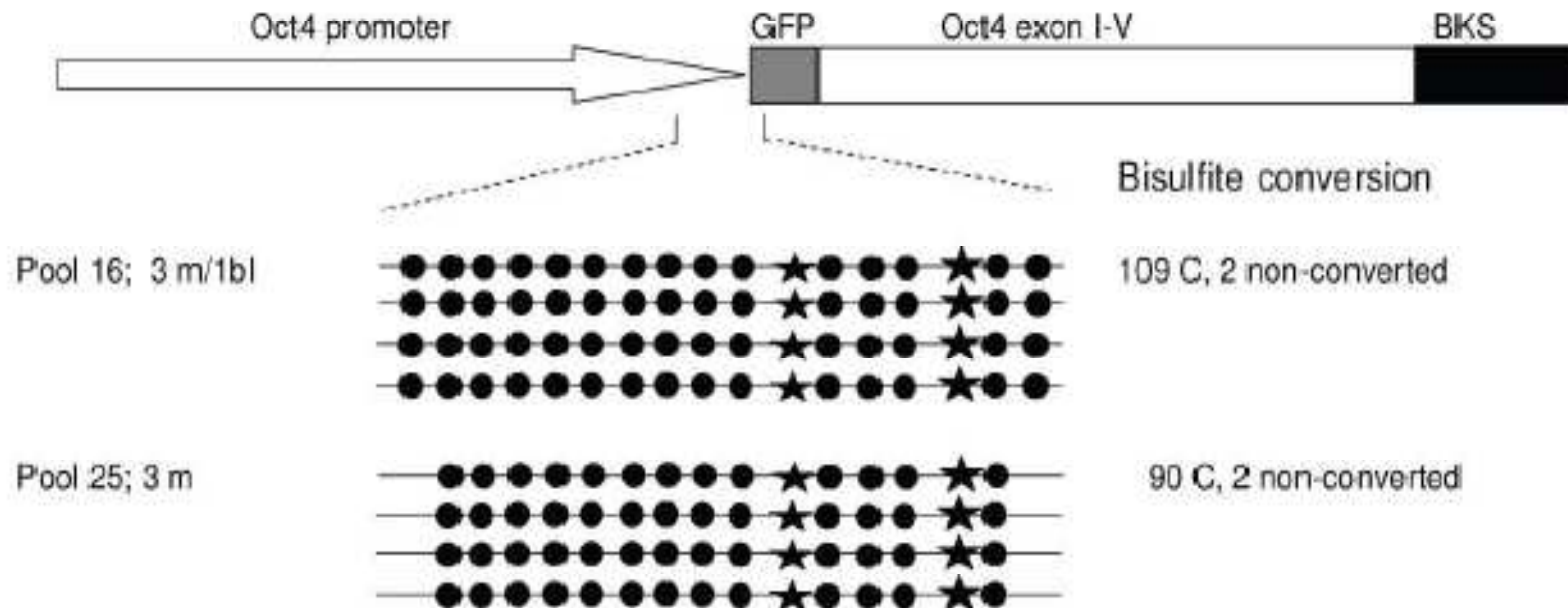


Figure 4. Methylation of the plasmid-encoded Oct4-eGFP promoter region. Schematic outline of the Oct4-eGFP construct. For clarity, the linearized form is depicted. The plasmid was methylated by *SssI* and injected into the cytoplasm of bovine zygotes. After 6 days of culture, morulae and early blastocysts with homogenous eGFP expression were taken for recovery of DNA and bisulfite sequencing. Two replicates showed completely methylated CpG dinucleotides in the analyzed amplicons (filled dots). In addition, non-CpG dinucleotides were 5-cytosine-methylated at two positions (filled stars). These sites are consensus sequences (CCWGG) for the bacterial DNA methylase Dcm.

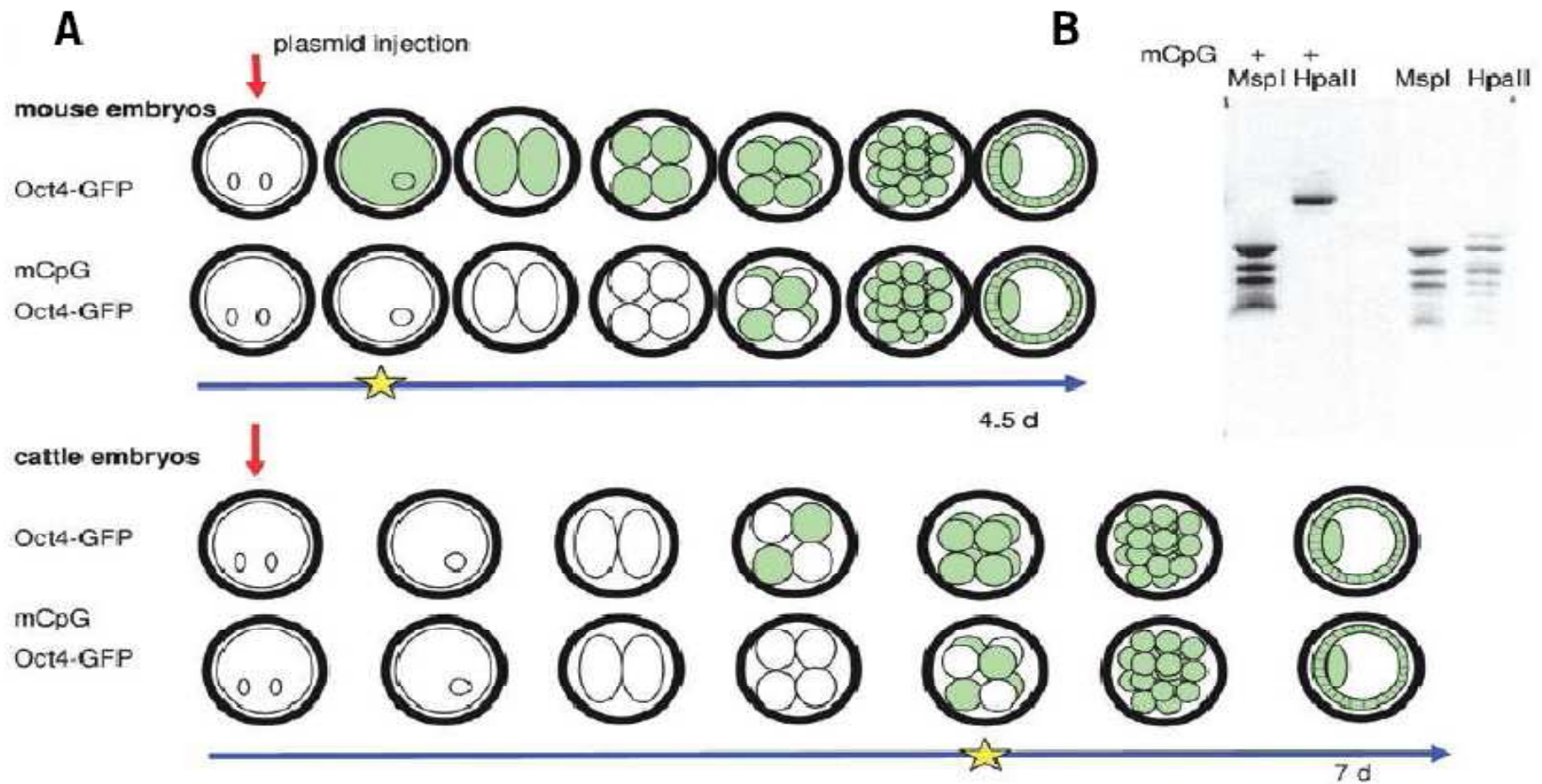


Figure 3. Onset of Oct4-eGFP expression coincides with major embryonic genome activation and is delayed by CpG methylation. (A) The Oct4-eGFP plasmid was injected in an unmodified or CpG-methylated version, into zygote stages of mouse and cow. The onset of eGFP expression (green blastomeres) was recorded by fluorescence microscopy in daily intervals; the summarized data are depicted. Note that murine embryos developed within 4 days to blastocysts, whereas bovine embryos needed 7–8 days. Stars (yellow) indicate the species-specific time points of major genome activation. (B) Verification of in vitro methylation of the Oct4-eGFP plasmid. The unmodified and the methylated (mCpG) plasmids were digested by the methylation-sensitive endonuclease *HpaII*. In parallel, digests with the methylation-insensitive isoschizomere, *MspI*, were performed.

LCR, Isolateurs, MAR...

- LCR (locus control region): ensemble de régulateurs distaux qui contrôlent l'expression coordonnée d'un groupe de gènes (locus des globines): situation rare.
- Isolateur: région qui s'oppose aux actions activatrices ou inhibitrices de régulateurs sur les promoteurs (isolateur \neq silencieux ou enhancer)
- Ouvreur de chromatine: région capable d'induire ou de maintenir localement la chromatine sous forme décondensée (euchromatine) indispensable pour la transcription
- MAR (matrix attached region): région le plus souvent riche en AT et participant à l'ouverture locale de la chromatine via l'action de topoisomérases (ne favorise généralement pas l'expression des transgènes)

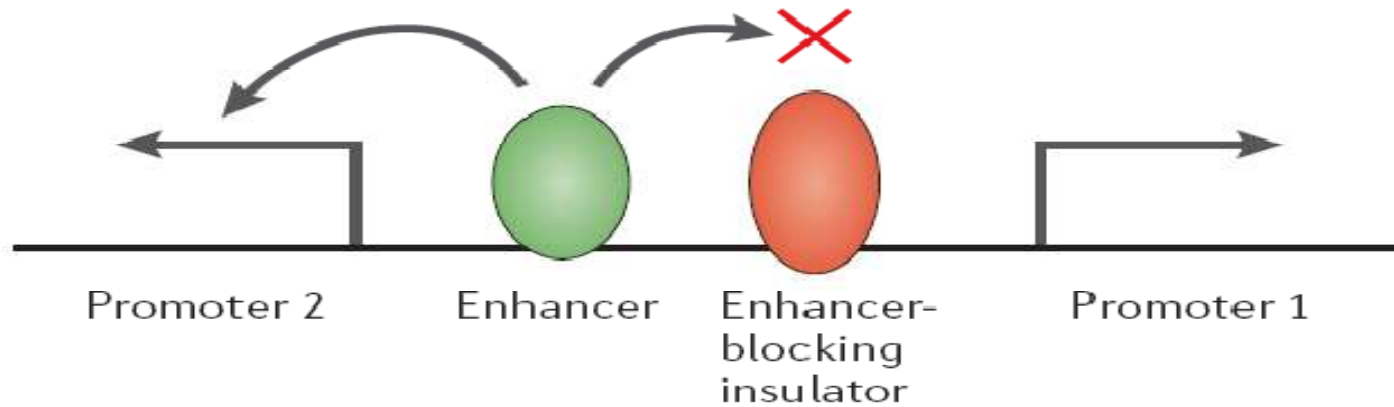
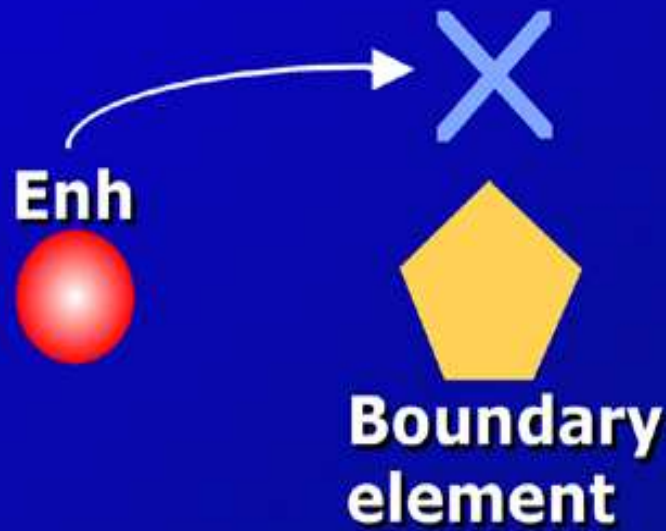


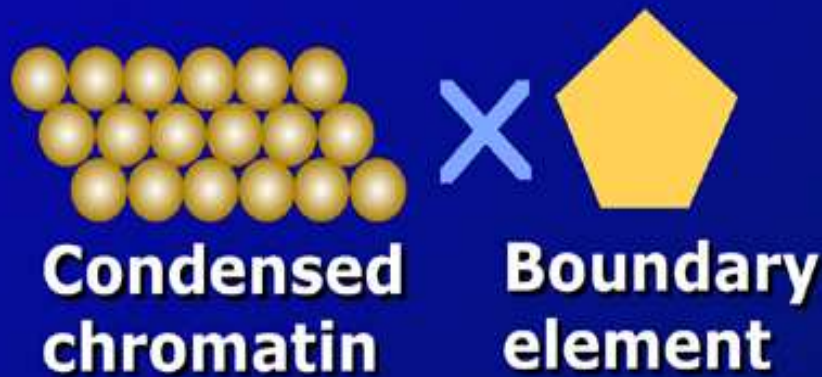
Figure 1 | Testing for enhancer-blocking insulator function. An enhancer-blocking insulator is expected to interfere with enhancer–promoter communication in a position-dependent manner. An enhancer-blocking insulator will block transcriptional activation only when it lies between a promoter and an enhancer (as in the case of promoter 1); in other situations (such as for promoter 2), activation is not blocked. A transcriptional repressor, by contrast, would reduce the level of transcription from both promoters when placed in the same position. In the example shown, the test construct contains both the experimental enhancer–promoter-1 and control enhancer–promoter-2 pairs; in other cases they can reside in separate transgenes.

a



Active locus

b



Active locus

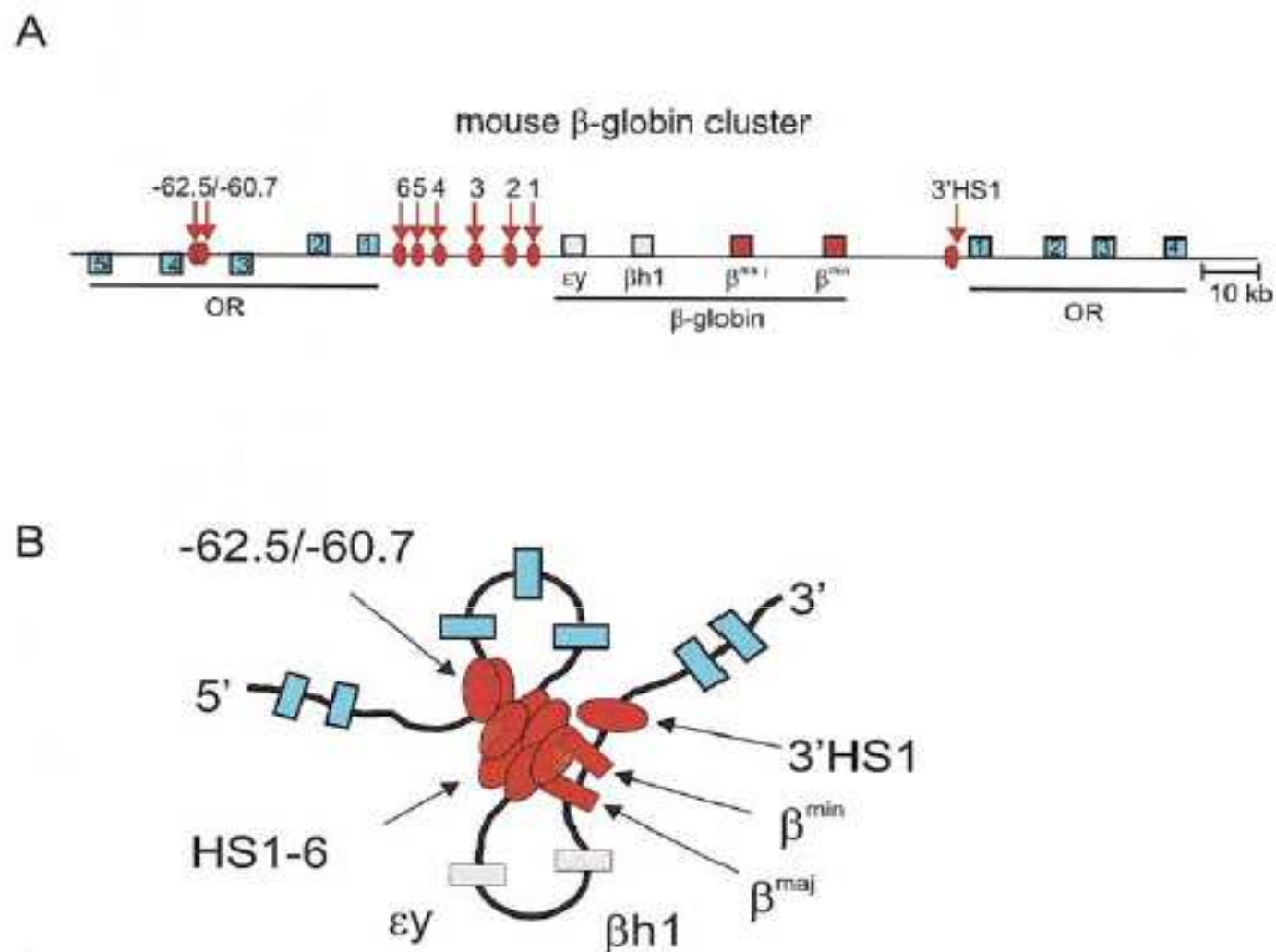
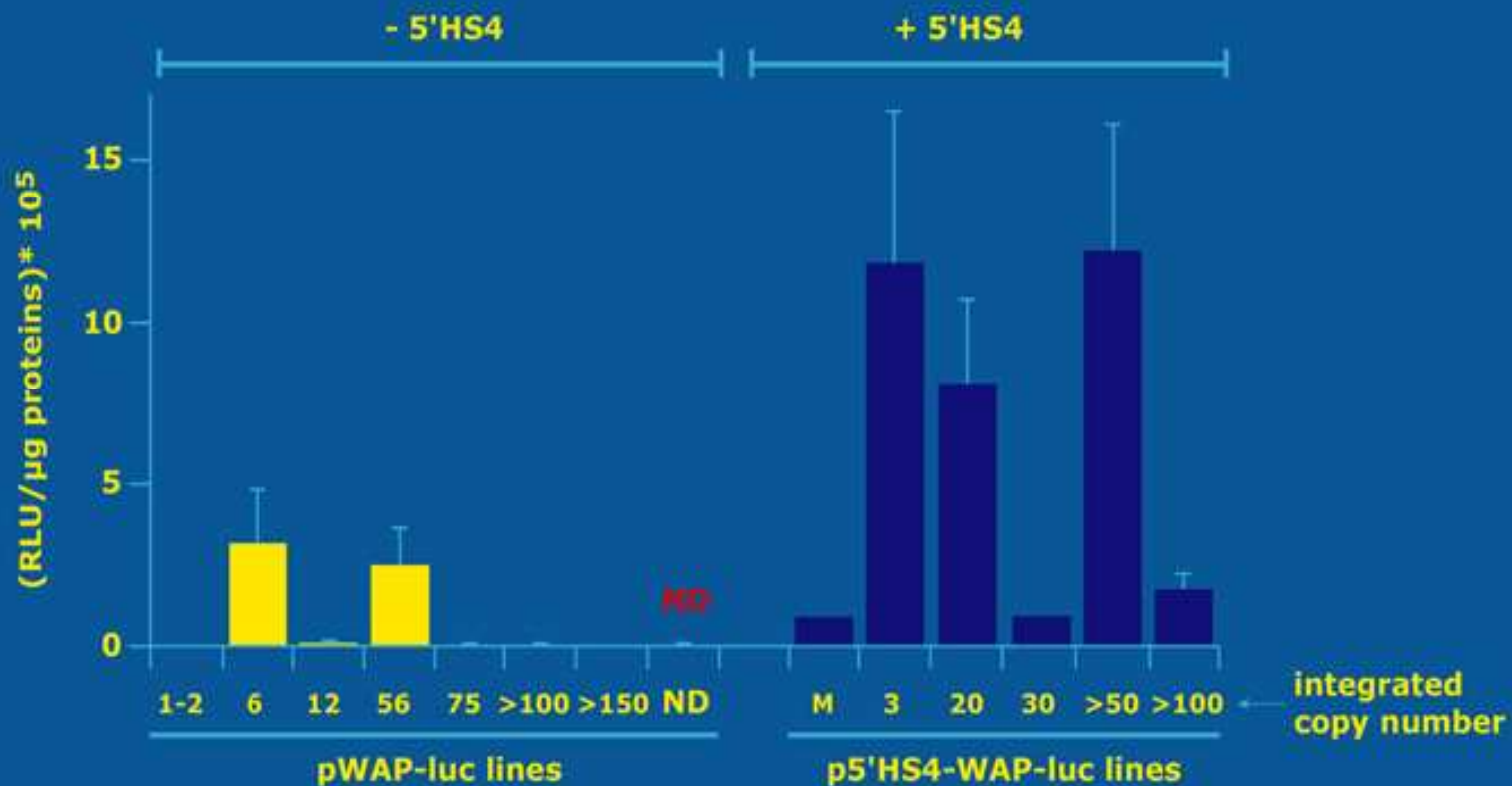


Figure 2. Spatial clustering of active genes and hypersensitive sites in the mouse β -globin locus. (A) Schematic presentation of the murine β -globin locus. Red arrows and ellipses depict the individual HSs. The active globin genes (β^{maj} and β^{min}) in the 14.5 dpc fetal liver are indicated by red boxes, the inactive genes ($\epsilon\gamma$ and βh1) are gray. The blue boxes indicate the olfactory receptor (OR) genes (5'OR1-5 and 3'OR1-4). (B) Clustering of active genes and hypersensitive sites in the mouse β -globin locus. Red indicates the active regions (hypersensitive sites and active genes) of the locus forming a hub of hyperaccessible chromatin (ACH). The inactive regions of the locus loop out, with the inactive βh1 and $\epsilon\gamma$ globin genes on one loop, and the 5'OR 1-3 genes on another loop. The interactions in the ACH would be dynamic in nature, in particular with the active genes (β^{maj} and β^{min}) which are alternately transcribed.

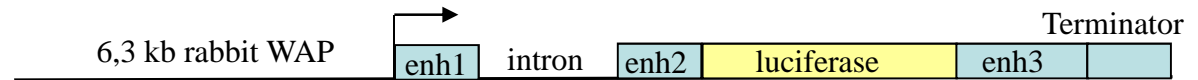
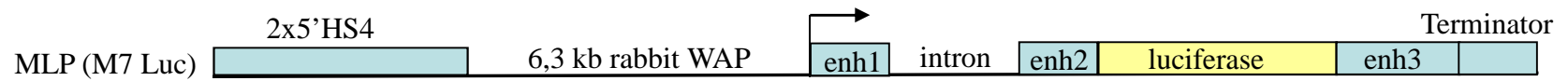
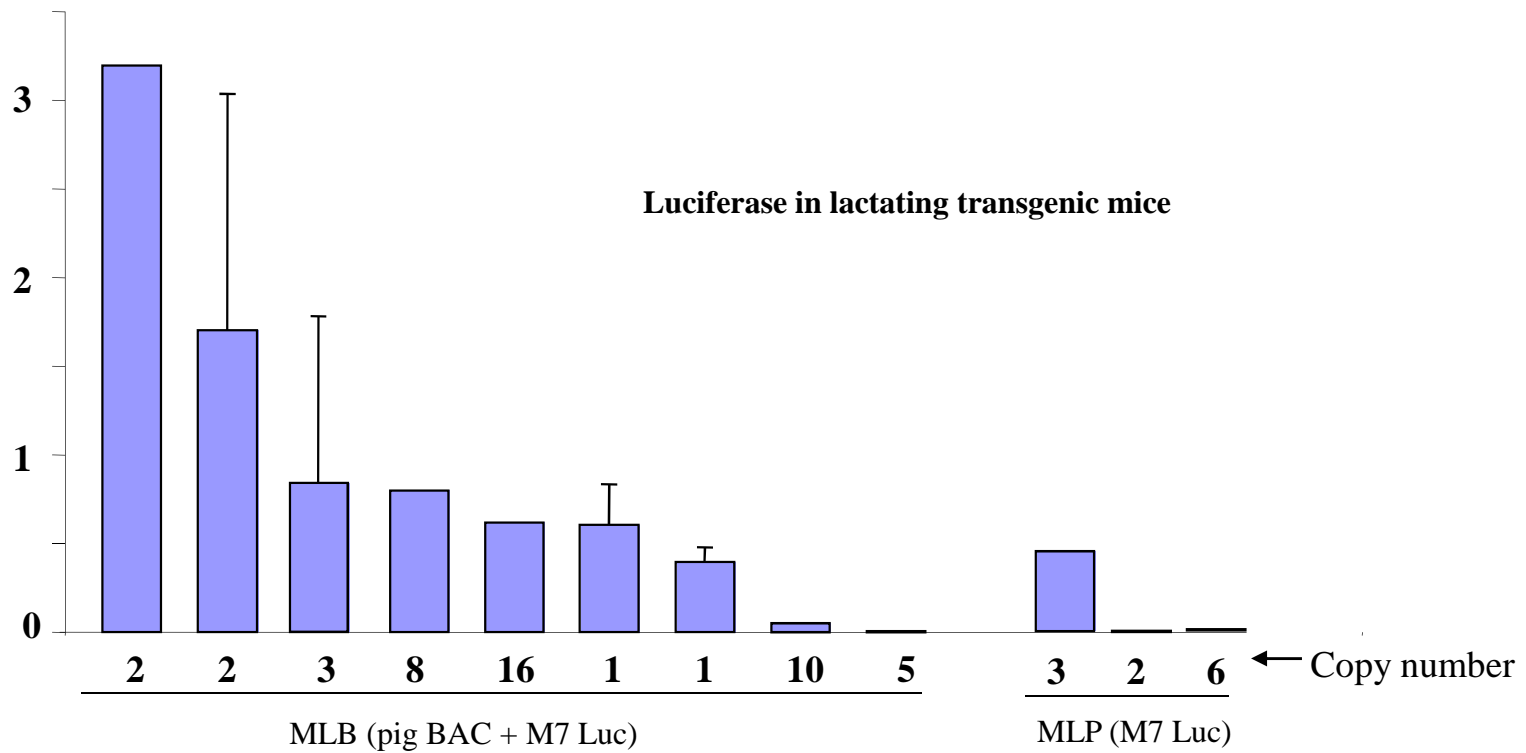
EFFECT OF 5'HS4 ELEMENT ON TRANSGENE EXPRESSION IN THE MAMMARY GLAND OF LACTATING ANIMALS



Conclusions :

- 5'HS4 decreases transgene extinction
- 5'HS4 enhances expression level
- 5'HS4 does not render expression dependent on copy number

Luciferase units/ug protein for one copy
(10⁻⁵)



MLB (pig BAC + M7 Luc)

Pig BAC

RAMP3

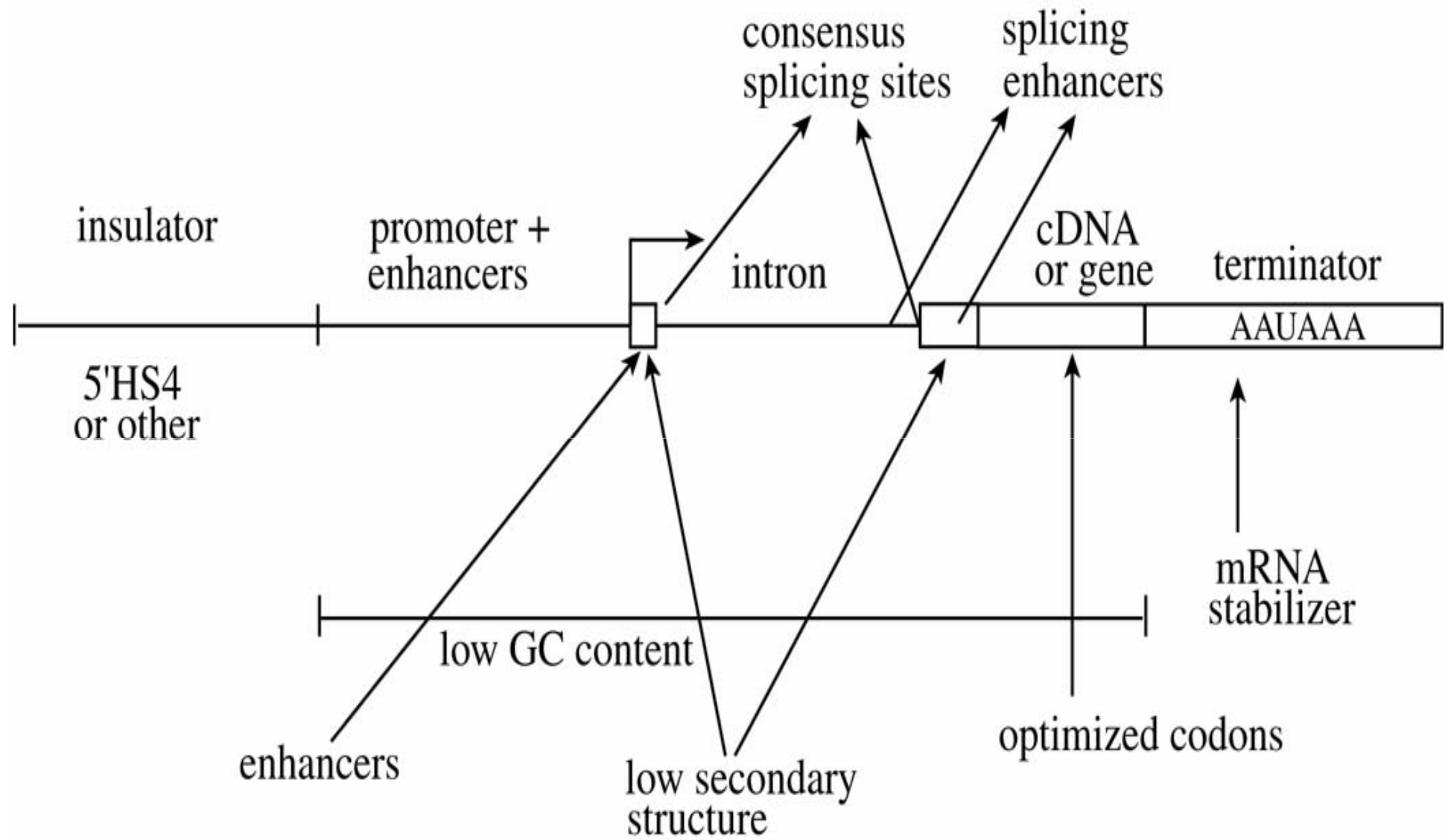
Pig WAP

X



Vecteurs contenant de longs fragments d'ADN (BAC)

- Difficulté relative des constructions de gène
- Fragilité mécanique des BAC
- Apport de régions génomiques indésirables (gènes)
- Réarrangements des BAC avant ou après transfert (régions répétées)
- Expression des transgènes souvent non totalement indépendante du site d'intégration et non dépendante du nombre de copies (non théoriquement attendue)
- Les BAC favorisent l'expression des transgènes mais sont difficilement utilisables en transgénèse pour étudier les éléments régulateurs des gènes



What to do if your transgene does not work well?

1. Evaluate the efficiency of your construct by transfecting it into cultured cells in which the promoter of your construct is active.
2. Make sure that the sequence of your construct is this you expected.
3. Make sure that a part of the coding sequence of your construct is not deleted after a cryptic splicing. This can be seen by a Northern blot or by RTPCR. If so, suppress (delete or mutate) the cryptic splicing (donor and acceptor) site(s) from your construct.
4. Add at least one intron preferably upstream of the cDNA to avoid NMD (see below). Choose introns having good splicing consensus sequences and splicing enhancers (*12*). The second intron of the rabbit β -globin gene is recognized as one of the good introns for transgenes.
5. Make sure that the mRNA coded by the transgene is not degraded by a nonsense mediated decay (NMD) mechanism. This occurs when the donor splicing site of the intron located downstream of the translated region is farther than 50 nucleotides from the termination codon (*13*).
6. Make sure that the 3'UTR does not contain an AU rich region with the AUUUA motif which induces an mRNA degradation in quiescent cells (*14*).
7. Use short 5'UTR containing not less than 80 nucleotides and being preferably AU rich to avoid the formation of stable GC rich secondary structure. The 5'UTR must not contain initiation codons within the consensus Kozak sequence.
8. Make sure that the initiation codon is within the Kozak consensus sequence **GCCA/GCCAUGG**.
9. Reduce the overall GC content of the construct and particularly the CpG motifs in the region preceding and following the transcription start point (*10*).
10. Add one or preferably two copies in tandem of the 5'HS4 insulator from chicken β -globin locus upstream of the promoter-enhancer region and optionally after the transcription terminator (*10,11*).
11. Use a strong transcription terminator, e.g. from rabbit or human β -globin genes or from human or bovine growth hormone genes.
12. Add mRNA stabilizer such as this present in the 3'UTR of α -globin gene (*15*).
13. Eliminate the sequences of the transcribed region of the construct (mainly in the 3'UTR) with may be recognized by natural miRNAs of the transgenic host.
14. Use as vectors long genomic DNA fragments cloned in BAC (bacterial artificial chromosome) containing the promoter chosen to express the transgene and introduce your construct (without any promoter) or your cDNA into the BAC after the promoter, for example after the first intron (*4*).
15. In bicistronic mRNA, put preferably the IRES (internal ribosome entry site) 80 nucleotides after the termination codon of the first cistron to favour the expression of the second cistron (*16*).
16. Optimize codon usage if the cDNA is not a mammal. This modification and others in the construct may require a complete chemical synthesis of the cDNA.

Springer Protocols

Methods in Molecular Biology 537

Rat Genomics

Methods and Protocols

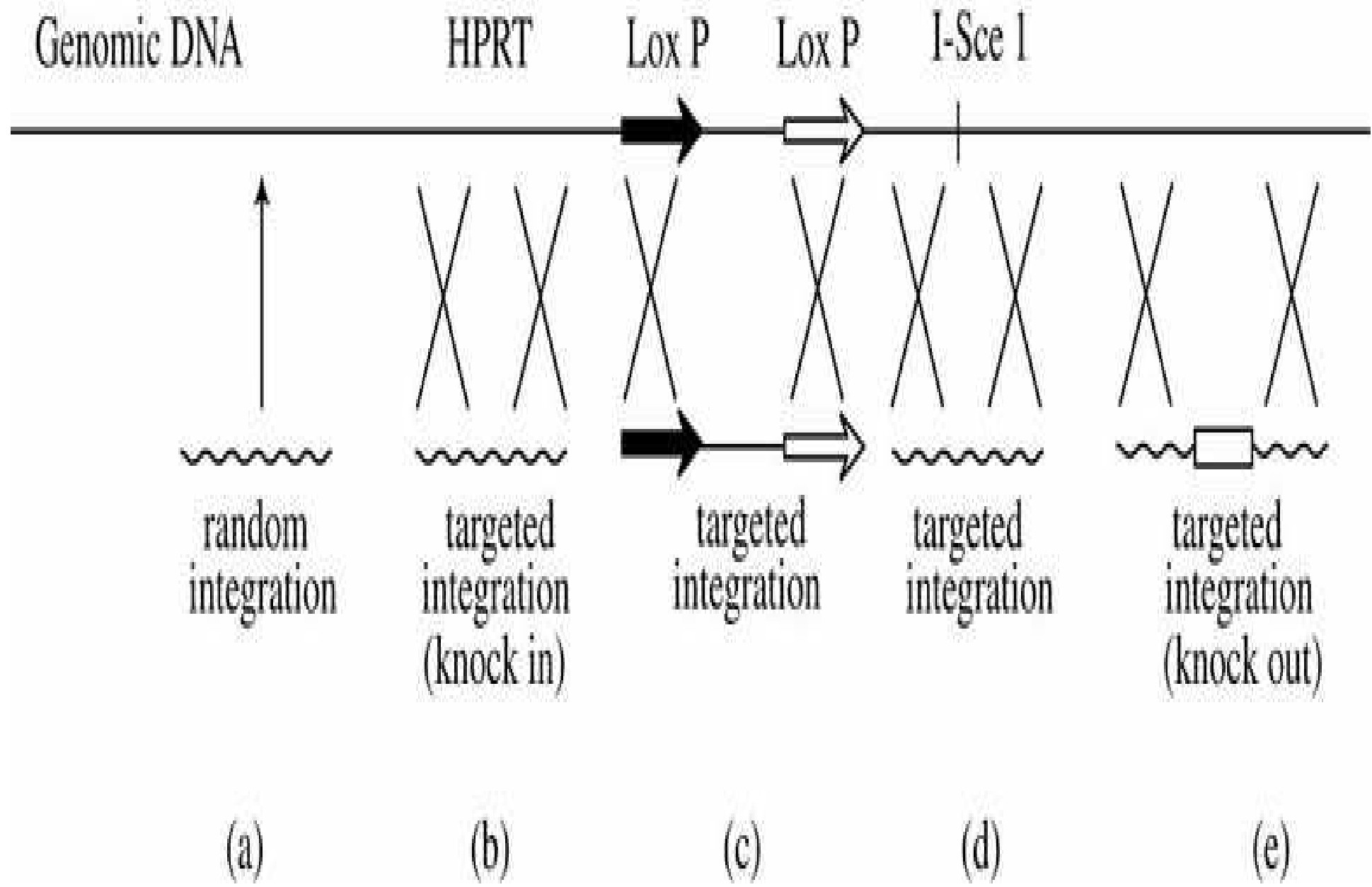
Edited by
Ignacio Aneón

 Humana Press

Chapter 4

Design of Expression Cassettes for the Generation of Transgenic Animals (Including Insulators)

Louis-Marie Houdebine



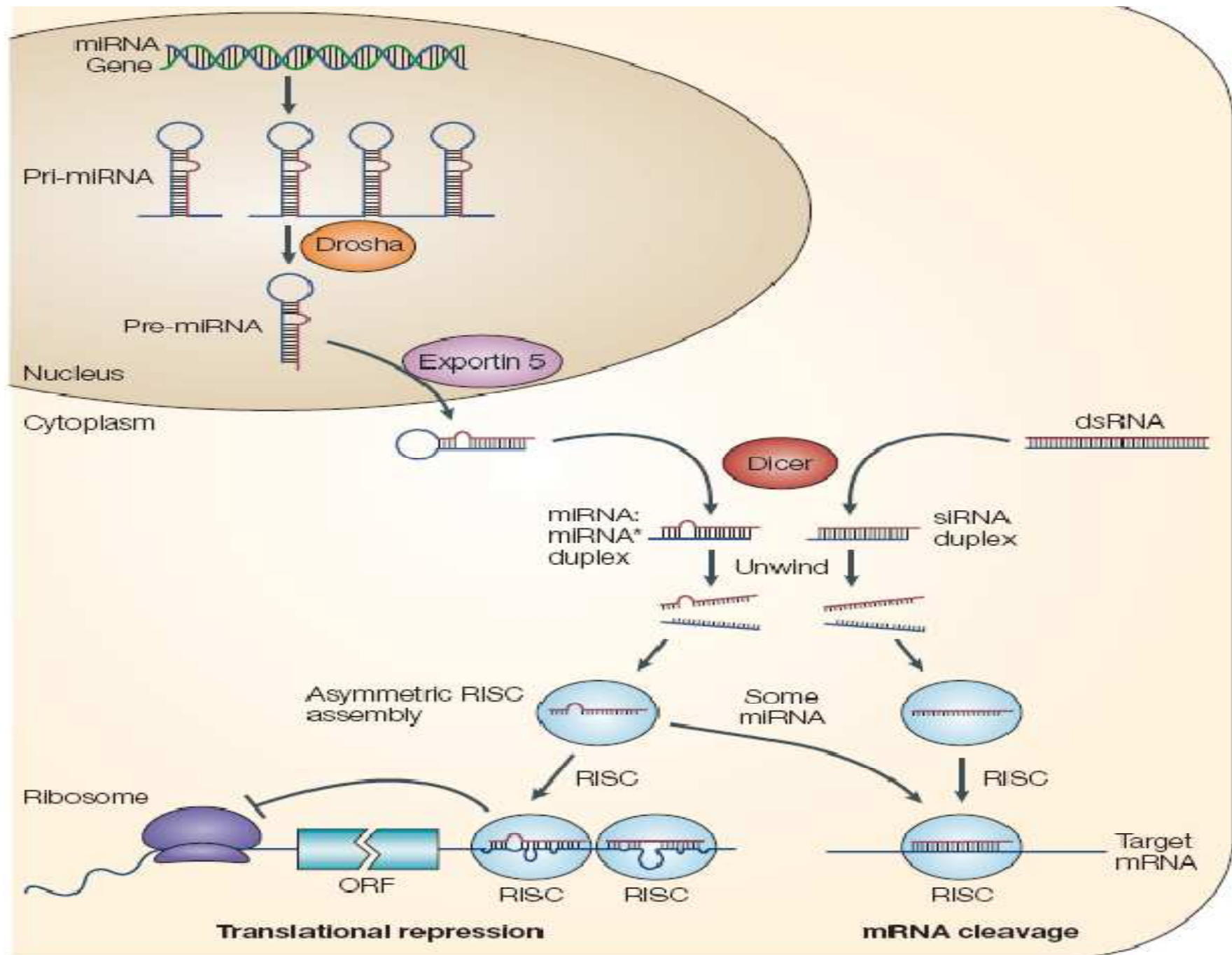
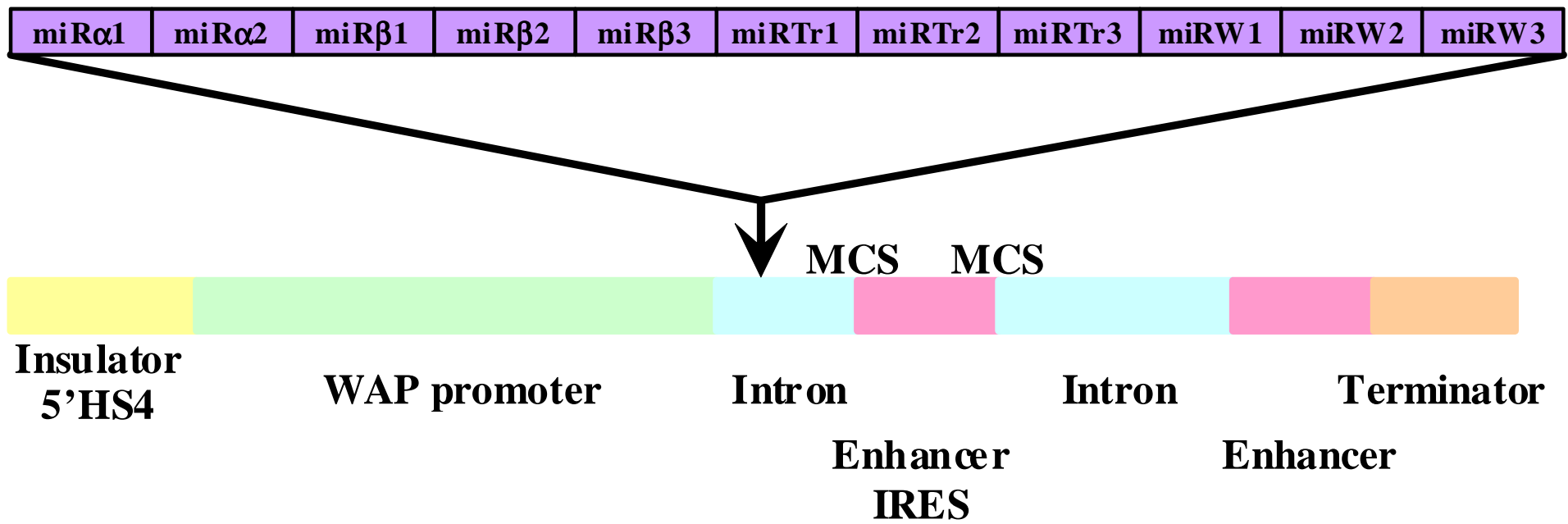
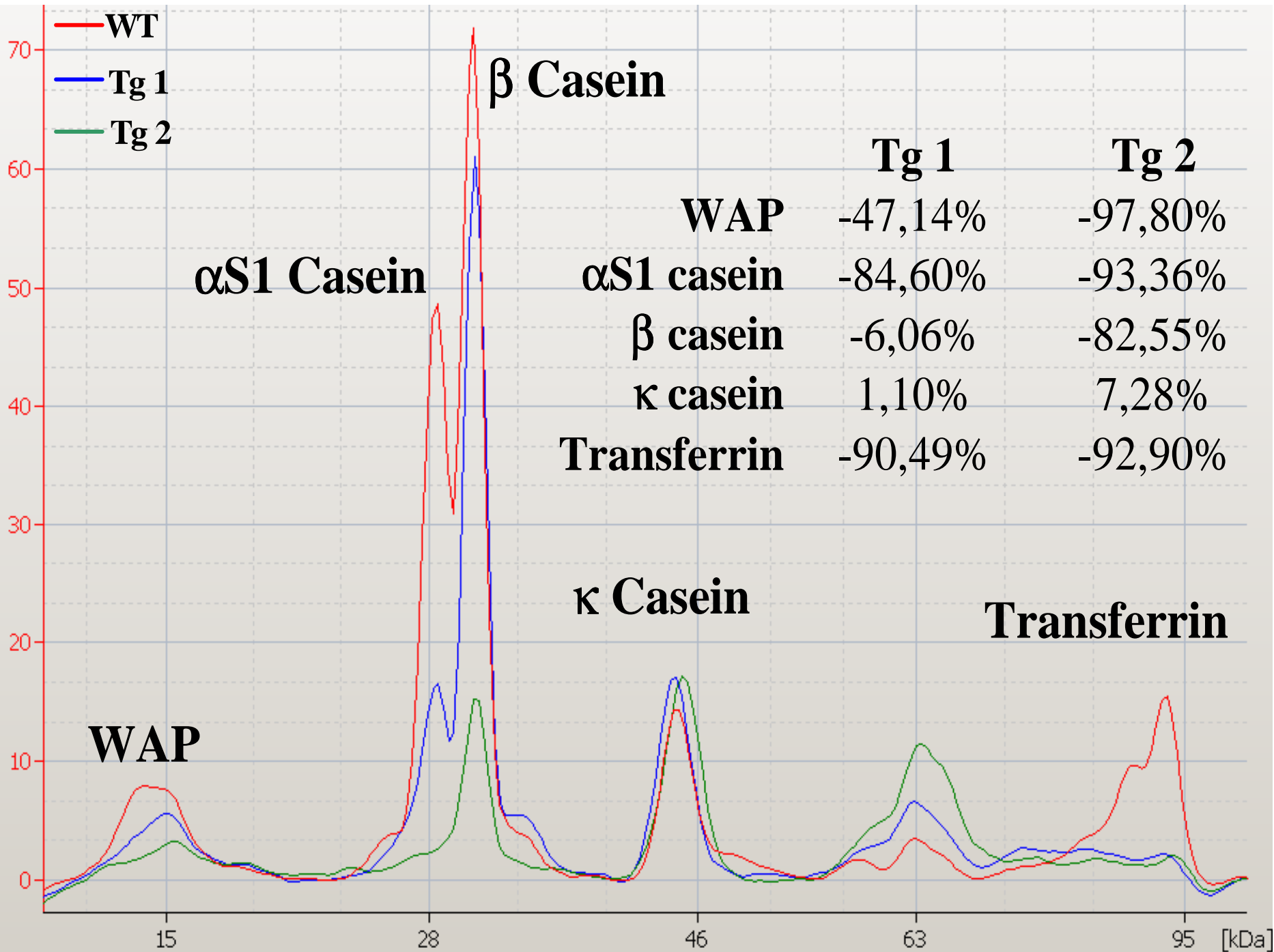
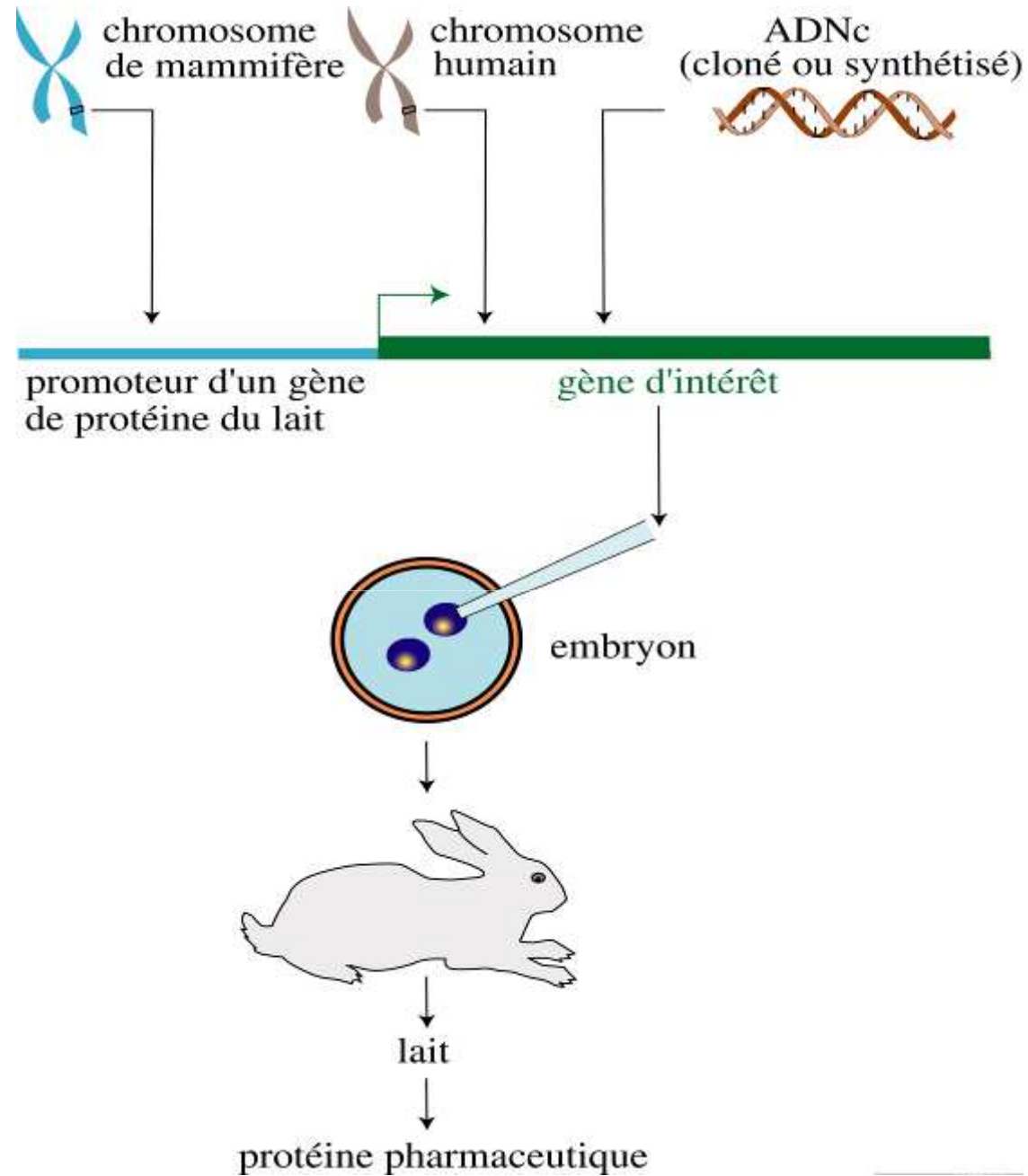


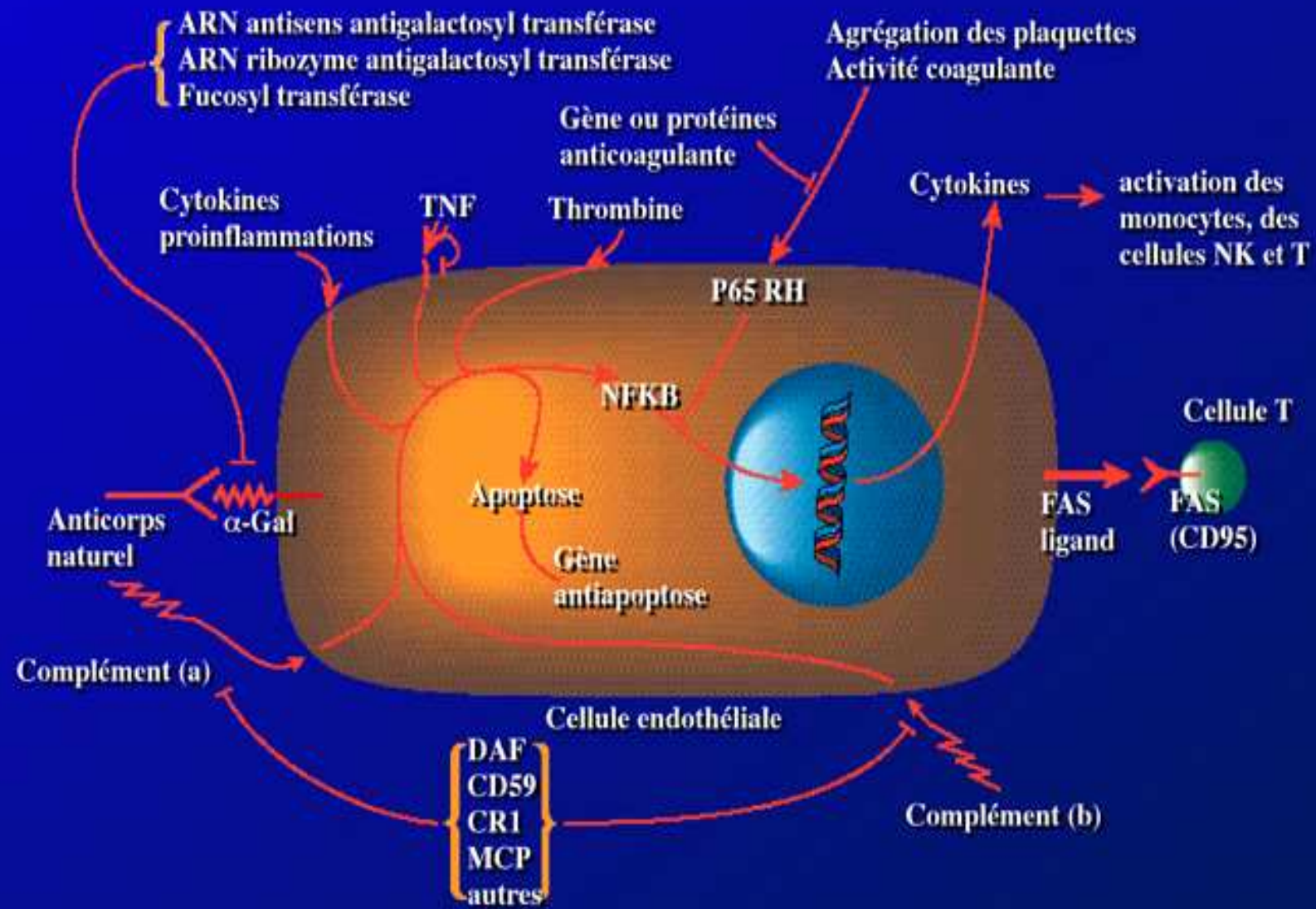
Figure 2 | **The current model for the biogenesis and post-transcriptional suppression of microRNAs and small interfering RNAs.** The nascent pri-microRNA (pri-miRNA) transcripts











FARM ANIMALS AS BIOMEDICAL MODELS

Obesity

Diabetes

Aging

Cardiovascular disorders

Infectious disease

Neurobiology

Epigenetics & environment

Ovarian cancer

Nutrition

Immunology

Genomics

Therapeutics

Ophthalmology

Reproduction

Research areas that could potentially be advanced by using farm animals as biomedical models. For appropriate farm animal models for specific diseases and references, see www.adsbm.msu.edu.

Obtention d'animaux génétiquement modifiés pour améliorer les productions animales

- * Accélération de la croissance des poissons (saumons)
- * Résistance aux maladies: pertes réduites, meilleur bien-être animal, moindre utilisation d'antibiotiques, réduction des zoonoses (mammites, cécropine chez les poissons, infections virales, prions PrP)...
- * Amélioration de la qualité du lait: moins de lactose et de protéines allergènes, transport de protéines anti-pathogènes (anticorps)
- * Amélioration de la qualité de la viande: plus de lipides polyinsaturés de type oméga 3
- * Réduction de la pollution: porcs sécrétant de la phytase dans leur salive

CAST[®] Issue Paper

Number 43
August 2009

**Animal Productivity and Genetic Diversity:
Cloned and Transgenic Animals**

Animal Agriculture's Future through Biotechnology, Part 8



BIO GUIDANCE

ON

GENETICALLY ENGINEERED ANIMAL

STEWARDSHIP

Draft; EFSA Background Paper on transgenic farm animals

1. Summary

2. Introduction

3. Transgenic technologies for farm animals

3.1 Gene transfer

3.1.1 In mammals

3.1.2 In poultry

3.1.3 In fish

3.1.4 In insects

3.2 Gene construction

3.3. Emerging transgenic technologies

3.3.1 Lentiviral mediated transgenesis

3.3.2 Conditional transgenesis in farm animals

3.3.3 Use of pluripotent cell lines

3.3.4 Spermatogonial transgenesis

3.3.5 RNA interference mediated gene knock down

3.3.6 Transgenics without transgenes

4. Applications of transgenic domestic animals

4.1 Basic research

4.2 Biomedical applications

4.2.1. Production of pharmaceutical proteins

4.2.2 Antibody production in transgenic animals

4.2.3 Blood replacement

4.2.4 Xenotransplantation of porcine organs to human patients

4.2.5 Farm animals as models for human diseases

4.3 Transgenic animals in agriculture

4.3.1 In mammals

4.3.2 In poultry

4.3.3 In fish

4.3.4 In insects

4.4 Transgenic pets

5. Health and welfare of transgenic farm animals

6. Safety aspects

7. References



Pegasus / part of LEI

pegasus



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[Contact](#)

[Project description](#)

[Project structure](#)

Pegasus



News

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Calendar

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Welcome

PEGASUS "Esse est Percipi"

PEGASUS is a collaborative project financed by the 7th Framework Programme FP7, KBBE 2008 GA 226465, European Commission, DG Research

PEGASUS: Public Perception of Genetically modified (GM) Animals – Science, Utility and Society

The general aim of PEGASUS is to provide policy support regarding the development, implementation and commercialization of GM animals, and derivative foods and pharmaceutical products. The results will contribute to the FP7 KBBE by integrating existing social, (including existing public perception) environmental and economic knowledge regarding GM animals.

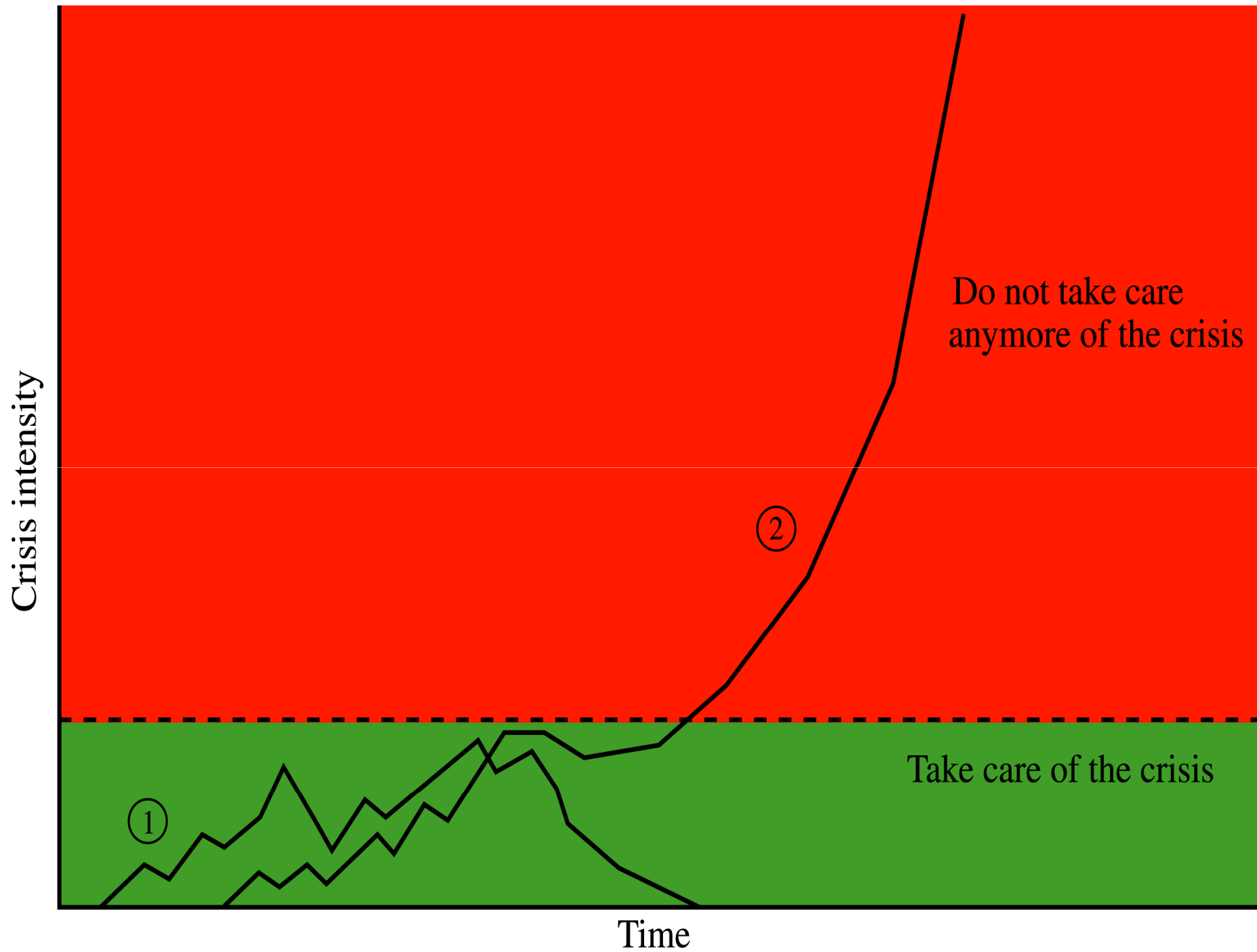
EU PEGASUS Project: Case Study

- 1. Title of the project:**
- 2. Transgenic Animals:**
- 3. Aim of the project**
- 4. Techniques used:.**
- 5. Proof of concept:**
- 6. Collaboration with private companies:**
- 7. Intellectual property, patents:**
- 8. Degree of achievement:**
- 9. Expected time for marketing:**
- 10. Expected economic impact:**
- 11. Possible impact on environment:.**
- 12. Ethical aspects:**
- 13. Contacts with regulation agency:**
- 14. Acceptability by consumers and public opinion:**
- 15. Debate with consumers, NGOs:**

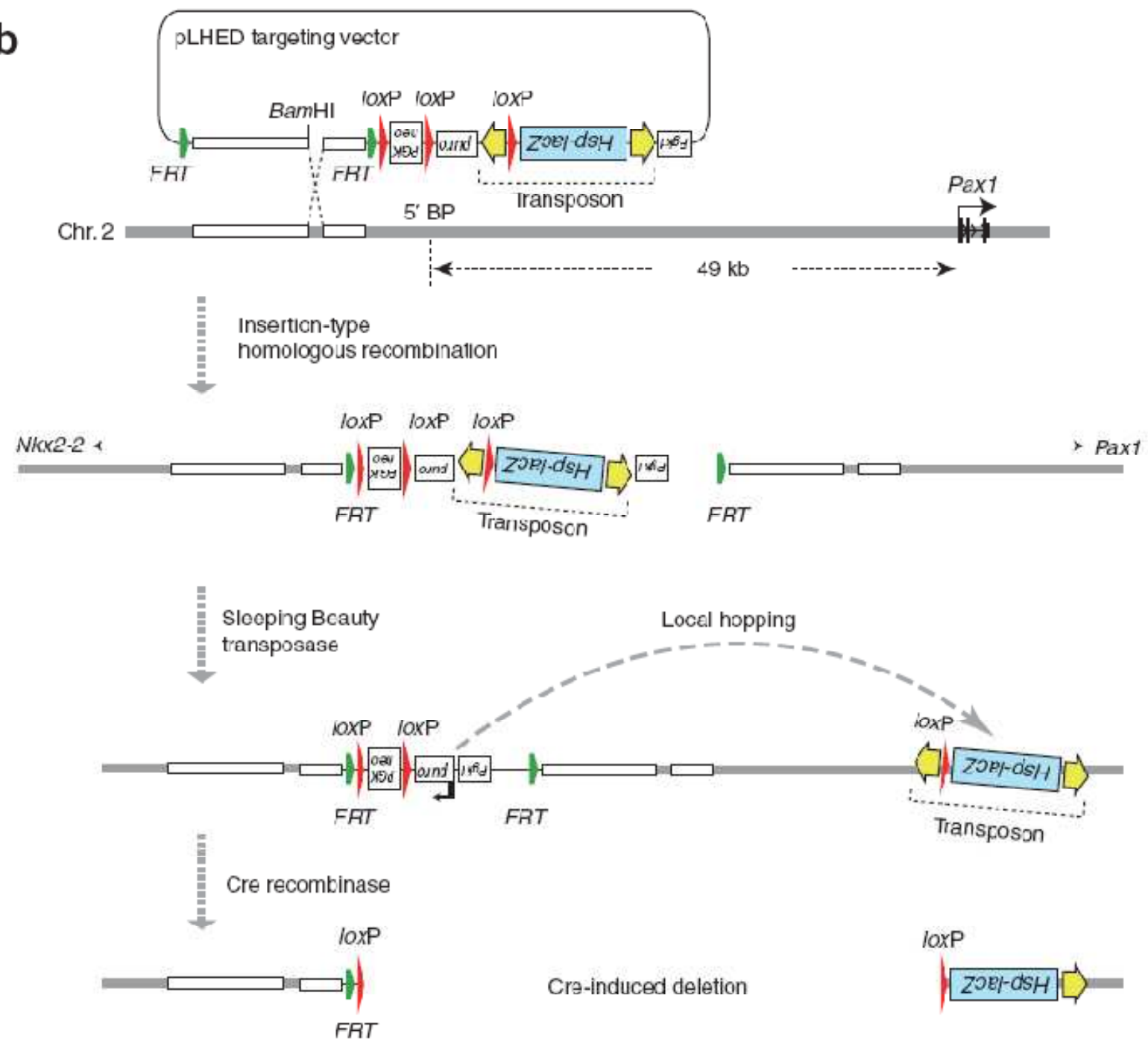
Acceptabilité des OGM

Conclusions d'une étude très approfondie réalisée dans 10 de l'UE et financée par l'UE

- 1) La principale limite à l'achat d'OGM est leur absence des étagères des supermarchés
- 2) L'achat d'OGM dépend de la décision des gérants de supermarché de les mettre en vente
- 3) La réponse à la question « les consommateurs achètent ils des OGM » est: oui lorsqu'on leur offre la possibilité



b



From DNA microinjection to iPS and ZFN

Louis-Marie Houdebine

Transgenic Res

DOI 10.1007/s11248-009-9325-5

MEETING REPORT

Meeting Report: UC Davis Transgenic Animal Research Conference VII

Granlibakken Conference Center, Tahoe City, California, August 17–21, 2009

Louis-Marie Houdebine

Utilisation des siRNA: Recommandations

- **Dessiner les séquences des shRNA en utilisant les règles consensus (riche GC en 5'P et riche en AU et 3'OH, taille de la boucle...)**
- **Rechercher les séquences homologues aux shRNA dans les génomes pour éviter les ciblage erratiques**
- **Rechercher dans les ARNm cibles les régions cibles ayant les plus faibles structures secondaires**
- **Introduire le gène de shRNA dans un vecteur PolIII (U6 ou H1)**
- **Tester les effets des des siRNA produits dans des cellules en mesurant l'inactivation de l'ARNm ciblé**
- **Garder de préférence les shRNA ayant des effets inhibiteurs intenses aux plus faibles concentrations (pour éviter les effets secondaires)**
- **Construire les vecteurs pour l'expression des transgènes: vecteurs contenant les gènes de shRNA ajoutés dans lentivirus, plasmides...**